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Embryonic Stem Cells

TEC-1 CHARACTERISATION OF PORCINE EMBRYONIC CELLS FROM DAY 11 EMBRYONIC DISCS CULTURED IN SERUM-FREE MEDIUM

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Day-11 porcine embryonic discs express cell phenotype markers typical of undifferentiated murine embryonic stem cells (ESCs): they are positive for the stage specific marker TEC-1/SSEA-1 (a potential marker of totipotency), but are negative for cytokeratin 8/18. However, culture of such discs in serum-containing medium induces partial differentiation whereby expression of TEC-1 disappears; whereas, cytokeratin expression becomes evident (Wianny et al., 1995, Soc. Franç. Étud. Fert. 34e Réun., Montpellier, Abst. C2). This differentiation may be induced by the growth factors and retinoids contained in serum. Hence, a serum-free medium was developed to re-examine TEC-1 expression patterns in primary cultures of porcine embryonic disc cells derived from day-11 blastocysts.

Embryonic discs were microsurgically dissected free of trophectoderm and endoderm and cultured on a fibronectin substrate (1 μg/cm^2) in DMEM/F12 medium supplemented with 10 μg/ml insulin, 5.5 μg/ml transferrin, 6.7 ng/ml selenium, 0.1 mM β-mercaptoethanol and either 10 or 100 ng/ml human leukaemia inhibitory factor (LIF): addition of 100 ng/ml LIF to this medium permitted 24/44 (54.5%) of attached embryonic discs to continue to expand after the third day in culture; whereas, 0/26 (0%) of discs cultured in only 10 ng/ml LIF survived, as all detached from the substrate at this time. Colonies cultured in 100 ng/ml LIF were stained for TEC-1 from the 3-5th day of culture. Compared to typical colonies cultured in the presence of serum, remarkably different TEC-1 expression patterns and cellular morphologies were observed in serum-free medium. Three major cell phenotypes could be identified: a) cells possessing a high nucleo-cytoplasmic ratio that appeared to be directly derived from the embryonic disc that were variably stained for TEC-1. Such cells appeared to be differentiating into two other cell types: b) epithelial-like polygonal cells staining both positively and negatively for TEC-1, so as to produce a mosaic-type staining pattern, c) cells of low nucleo-cytoplasmic ratio that formed rapidly expanding populations that were negative for TEC-1. TEC-1 positive cell populations accounted for 32.3 ± 31% (mean ± sd) of the total area of the colonies (n=16). Individual addition of other growth factors (10 ng/ml basic fibroblastic growth factor (n=8 colonies), 100 ng/ml epidermal growth factor (n=18) or 100 ng/ml insulin-like growth factor-II (n=10)) together with LIF (100 ng/ml) did not increase the percentage area represented by the TEC-1 positive cell populations (24.9 ± 33, 31.3 ± 30, and 29.4 ± 28%, respectively, $P > 0.05$).

These results indicate: i) high concentrations of LIF are essential to maintain colonies in serum-free medium, ii) removal of serum (or specific serum components) permits continued expression of TEC-1 which, if analogous to murine ESCs, suggests a maintenance of totipotency in embryonic disc-derived cultured cells. Further examination of the growth factor requirements that can specifically expand these TEC-1 positive populations is required.

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